Physicochemical Characterization and Toxicity of Decursin and Their Derivatives from *Angelica gigas*

Bimit Mahat,[a,#] Jung-woo Chae,[a,#] In-hwan Baek,[d] Gyu-yong Song,[a] Jin-sook Song,[b] Seong-kwon Cho,[c] and Kwang-il Kwon*[,a]

[a] *College of Pharmacy, Chungnam National University; Daejeon 305–764, Republic of Korea:* [b] *Drug Discovery Platform Technology Team, Division of Bio-organic Science, Korea Research Institute of Chemical Technology; Daejeon 305–600, Republic of Korea:* [c] *Samnam Pharmaceuticals; Geumsan 312–802, Republic of Korea: and* [d] *Incrementally Modified Drug (IMD), Yuhan Research Institute; Gyeonggi-do 446–902, Republic of Korea.*
Received January 12, 2012; accepted April 23, 2012

Angelica gigas **NAKAI is used to treat dysmenorrhea, amenorrhea, menopause, abdominal pain, injuries, migraine, and arthritis. The present study provided a physicochemical and toxicological characterization of compounds in *A. gigas* NAKAI (decursin, decursinol angelate, diketone decursin, ether decursin, epoxide decursin and oxim decursin). Diketone decursin (173.16 μg/mL) and epoxide decursin (122.12 μg/mL) exhibited >100 μg/mL kinetic solubility after applying nephelometry, suggesting a highly soluble compound. The Student's *t*-test revealed significant differences in the pK_a ranges of the compounds by automatic titration from capillary electrophoresis ($p<0.05$). Diketone decursin, epoxide decursin and oxim decursin might be formulated into an oral dosage form (log*P*: 0–3) by an automatic titration analysis. A parallel artificial membrane permeability assay demonstrated permeability coefficients of $<10\times10^{-6}$ cm/s for all of the compounds, suggesting poor permeability. Ether decursin exhibited a toxic effect after being applied to mouse (NIH 3T3, EC_{50}: 57.9 μM) and human (HT-29, EC_{50}: 36.1 μM; Hep-G2, EC_{50}: 4.92 μM) cells. Additionally, epoxide and oxim decursin were toxic through acute oral toxicity (four and three deaths of Institute of Cancer Research (ICR) mice) and mutation toxicity testing by applying *Salmonella typhimurium* cells with and without S9. Although diketone decursin exhibited less permeability, it is potentially valuable pharmacological compound that should be investigated.**

Key words Institute of Cancer Research mice; diketone decursin; *Angelica gigas*; physicochemical; toxicology

A recent drug-development study reported that 46% of 121 new chemical entity compounds were unacceptable based on efficacy, and 40% were rejected due to safety issues. Thus, early- and late-phase drug discovery emphasizes compound optimization.[1)] Physicochemical analysis is fundamental during the early phase,[2)] and toxicology studies are conducted during the late phase.[3)] Physicochemical properties are evaluated to determine solubility, ionization constant, lipophilicity, and permeability of compounds,[4)] whereas toxicology studies are conducted to investigate acute toxicity, mutations, and cytotoxicity of compounds.[5–8)]

Predicting solubility is difficult due to a lack of reliable and reproducible solubility measurements.[9)] Additionally, insufficient solubility can affect the results of early high-throughput screening assays and the ability to achieve efficacious and toxicologically relevant exposures in animals.[2)] Kinetic solubility (the concentration of a compound at the time when an induced precipitate first appears in the solution) is a commonly used term in the field of pharmaceutics.[10)] The solubility of a compound at a given pH containing basic or acidic functional groups is always influenced by the ionization characteristics of the compound.[2)] Furthermore, the ionization of drugs affects binding at the active site, thereby influencing not only cytochrome activity but also therapeutic efficacy.[11)] Thus, the charge state dramatically affects lipophilicity and pharmacokinetic behavior, not to mention the affinity of drug-metabolizing enzymes and drug targets.[12)] Solubility and permeability, as well as other absortption, distribution, metabolism, excretion (ADME) properties such as protein binding and tissue distribution, are affected by lipophilicity.[2)] Finally, permeability is an important factor to determine transport through the gastrointestinal tract, penetration of the blood–brain barrier, and transport across cell membranes.[13)]

The identification of compounds capable of inducing mutations is crucial in a safety assessment, as mutagenic compounds can potentially induce cancer.[5,6)] The Ames test, which is conducted using *Salmonella typhimurium*, is a widely used bacterial assay for identifying chemicals that can produce gene mutations.[7)] Furthermore, acute toxicity testing helps to obtain information on the biological activity of a chemical and insight into its mechanism of action. Acute toxicity testing also identifies adverse change(s) immediately following a single or short period of exposure to a substance.[8)] Finally, cytotoxicity assays help to evaluate toxic mechanisms of compounds and have recently gained acceptance by discovery scientists to aid in the selection and design of chemical structures for further development.[3)]

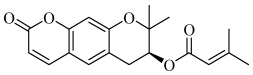
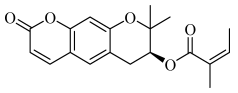
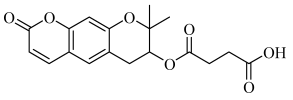
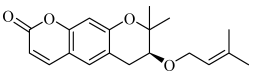
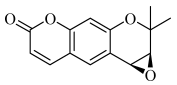
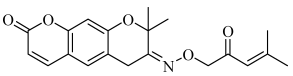
Angelica gigas NAKAI and its substances are well known for treating dysmenorrhea, amenorrhea, menopause, abdominal pain, injuries, migraine, and arthritis.[14)] Thus, many pharmacokinetic studies have been conducted regarding *A. gigas* NAKAI and its substances, which include decursin and decursin isomer (decursinol angelate).[14–16)] However, no study has considered decursin derivatives (diketone decursin, ether decursin, epoxide decursin, and oxim decursin), which could potentially be developed as therapeutic agents. The presence of these compounds can be confirmed based on molecular

The authors declare no conflict of interest.

[#] These authors contributed equally to this work.

* To whom correspondence should be addressed. e-mail: kwon@cnu.ac.kr

Table 1. Molecular Weight and Structure of Decursin and Their Derivatives

Compound	Molecular weight	Structure
Decursin (main component of *Angelica gigas*)	328.4	
Decursinol angelate (decursin isomer)	328.4	
Diketone decursin (decursin derivative)	346.3	
Ether decursin (decursin derivative)	314.4	
Epoxide decursin (decursin derivative)	244.3	
Oxim decursin (decursin derivative)	341.4	

weight and structure (Table 1).

The present study provides a physicochemical and toxicological characterization of the *A. gigas* Nakai compounds by applying recent techniques to examine the potential of these compounds as therapeutic agents.

MATERIALS AND METHODS

Materials The test compounds (decursin, decursinol angelate, diketone decursin, ether decursin, epoxide decursin and oxim decursin) were supplied by the medicinal organic chemistry laboratory of Chungnam National University (Daejeon, South Korea) at >98% chemical purity. All solvents were high-performance liquid chromatography (HPLC) grade and were purchased from Merck Co. (Darmstadt, Germany).

Animals Male Institute of Cancer Research (ICR) mice (weight, 25–40 g) and non-pregnant female ICR mice (weight, 20–35 g) were purchased from Orient Bio, Inc. (Seongnam, Korea) and maintained at the Korea Research Institute of Chemical and Technology (KRICT) Animal Care Center for 8 weeks. They were housed under controlled temperature and humidity conditions with an alternating 12 h light/dark cycle and free access to food and water. All animal care and housing procedures followed the KRICT Animal Care and Use Committee guidelines.

Sirius Analytical Instrument The apparatus to perform the solubility determinations was a Sirius GLpKa titrator fitted with a pH electrode (combination Ag–AgCl), and a Sirius D-PAS spectrometer with a bifurcated fiber optic dip probe (Hellma, Southend-on-Sea, Essex, U.K.) with a path length of 1 cm. The apparatus was controlled *via* a computer running RefinementPro and CheqSol software (Sirius Analytical Instruments, Ltd., Forest Row, East Sussex, U.K.). All titrations were performed in 0.15 M KCl solution under an argon atmosphere at 25±1°C, using standardized 0.5 M HCl and 0.5 M KOH solutions. The pH electrode was calibrated titrimetrically in the pH range of 1.8–12.2.

Determination of Kinetic Solubility by Nephelometry The laser nephelometer used in this study was a NEPHELOstar (BMG Lab Technologies, Offenburg, Germany), and a 96-well microtiter plate (EG and G Wallac, Milton Keynes, U.K.) was installed in the instrument. Stock solutions of test compounds were prepared at 10 μM in 5% dimethyl sulfoxide (DMSO): 95% phosphate buffered saline (PBS) buffer manually as an optimum concentration.[17] All sample wells were scanned with an integration time of 0.1 s, and each plate was read vertically with a gain of 30 and a laser intensity of 90% to produce a raw count data per well. Finally, all raw data were processed using the BMG LABTECH NEPHELOstar Galaxy Evaluation software.[10,17]

Determination of pK_a by Capillary Electrophoresis The method was based on the guidelines provided by Avdeef and Testa[12] and Fuguet *et al.*[18] Studies were performed using an Agilent Technologies (Santa Clara, CA, U.S.A.) capillary electrophoresis system equipped with a diode-array spectrophotometer detector. A fused silica capillary of 50 μM i.d., 375 μM o.d., and 48.5 cm total length (40 cm to the detector) was obtained from Composite Metal Services (Ilkey, England) and used to carry out the experimental mobility determinations. The temperature of the capillary was kept at 25°C. About 10 μL of test compounds (50 μM) was injected hydrodynamically and was gathered at one end of the capillary. The applied voltage was 20 kV. Sample consumption was about 0.2 μg per injection. Detection was carried out at 214 nm. A pressure of 50 mbar was applied during the separation to speed the analysis. Sample species migrated according to charge and fluid drag. The practical range of the buffer pH in the capillary electrophoresis was 3–11.[19,20] Finally, all running buffers

and samples were filtered through a *nylon filter* with 0.45-μm pore size.

Determination of log$P$ and pK_a by Automatic Titration The method was based on the guidelines provided by Völgyi *et al.*[21] and by Krämer *et al.*[22] A titration of 50 μmol test compounds in 20 mL of 0.15 M KCl was performed on the Sirius analytical instrument using the above conditions to determine the pK_a. In log$P$ experiments, *n*-octanol/0.15 M KCl was between 0.0025 and 18. The aqueous volume was $\geq$5 mL. *n*-Octanol was saturated with 0.15 M KCl prior to dosing. Finally, pK_a and log$P$ were evaluated using the pK_a LOGP program from the Sirius analytical instrument, which has been described previously also.[23,24]

Determination of Permeability by Parallel Artificial Membrane Permeability Assay PAMPA A permeability by PAMPA was performed using 96-well hydrophilic filtration plates (Millipore, Bedford, MA, U.S.A.). Filter material was prepared by wetting each well of the filtration plate with 5 μL of artificial membrane solution (0.8% egg lecithin in *n*-dodecane). The donor and recipient solutions consisted of phosphate-buffered saline (pH 5.5 or 7.4) with 2% DMSO. The donor solution also contained 200 μM of the test compound. The PAMPA receiving plate was a Millipore GV hydrophilic filter plate containing 272 μL of blank phosphate buffered saline (pH 5.5 or 7.4), and the donor plate was a Dynex 96-well plate (Dynex, Ashford, Middlesex, U.K.) filled with 272 μL of donor solution. The stacked donor and receiving plates were incubated for 2 h at room temperature with gentle circular shaking, and then the compound concentrations in the receiving solutions were assayed by HPLC coupled with ultraviolent absorbance detection (235, 254 nm). The HPLC system consisted of a YMC AQ 4.6×50-mm column (YMC, Inc., Wilmington, DE, U.S.A.) using a mobile phase gradient of 80% acetonitrile/20% ammonium formate. Injection volume was 50 μL. The artificial permeation of test compounds was evaluated in solutions consisting of 0.5 N HCl (pH 1.5) or phosphate-buffered saline (pH 5.5 or 7.4) with 2% DMSO. The apparent permeability coefficient (P_{app}) for each test compound was calculated from the following equation:

$$P_{app} = C \times -\ln\left(1 - \frac{[\text{drug}_{\text{acceptor}}]}{[\text{drug}_{\text{equilibrium}}]}\right)$$

where

$$C = \frac{V_D \times V_A}{(V_D + V_A) \times \text{area} \times \text{time}}$$

where V_D and V_A are the volumes of the donor and acceptor compartments, respectively; area is surface area of the membrane multiplied by the porosity, and the equilibrium drug concentration is the concentration of test compound in the total volume of the donor and acceptor compartments.[13,25]

Acute Oral Toxicity Test substances were dissolved in 20% DMSO: 80% corn oil to a nominal working concentration of 100 mg/mL. The animals were allowed a 6-d acclimatization period before they entered the study. Each test compound was provided to eight animals (four males and four females) with weight as the limiting factor. The animals were housed singly during the first 48 h after dosing. Following the 48-h observation period, all animals were returned to their original groups, and the animals were housed in groups of eight for the remainder of the 14-d observation period. The animals were monitored for the first 30 min after dosing and twice daily on the day of dosing. Observations were done once daily thereafter for the remainder of the 14-d observation period. Test substances were administered by oral gavage using a stainless steel gavage needle. The animals were dosed with 10 mL/kg body weight of the supplied solution (based on a target concentration of 100 mg/mL). The day of dosing was designated as day 1, and animals were weighed at days 1, 7, and 14 (termination). All mice have same weight in day 1 of dosing. Animals were observed for survival and changes in weight. The mice were sacrificed by cervical dislocation at the end of the observation period.[26]

Mutation Sodium azide (NaN_3), 2 nitro fluorine ($C_{13}H_8NO_2$), and benzo(*a*)pyrene ($C_{20}H_{12}$) were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). *Salmonella typhimurium* strains TA98 and TA100 were provided by the Korea Collection for Type Cultures (KCTC, Seoul, Korea). Culture stocks were stored at −80°C until use. For all assays, an inoculum of a thawed permanent culture was added to 20 mL of 2.5% Oxoid nutrient broth #2 (Oxoid, Ltd., Cambridge, U.K.) and incubated in a shaking incubator (Vision Scientifics, Bucheon, Korea) for 12–14 h at 37°C with a shaking speed of 210 rpm, yielding 1–2×10^8 cells/mL. The lyophilized rat liver S9 fraction induced by Aroclor 1254 was purchased from Moltox (Boone, NC, U.S.A.). The S9 mixture was prepared as described previously by Gomes-Carneiro *et al.*[27] $C_{13}H_8NO_2$ was used as a positive control for *S. typhimurium* strain TA 98 without the S9 mix, and NaN_3 was employed as a positive control for *S. typhimurium* strain TA 100. $C_{20}H_{12}$ was applied as positive control in TA 98 and TA 100 for the S9 mix. DMSO was employed as negative control for both TA 98 and TA100 as well as for treatments with and without the S9 mix.

The *S. typhimurium* plate incorporation assay was employed according to Ames *et al.*[28] The mutagenicity assay was performed by incorporating test compounds with *S. typhimurium* strains TA 98 and TA 100, with or without the metabolic activation; 5000 μg per plate of test compounds was used. NaN_3 and $C_{13}H_8NO_2$ were used at 1 μg per plate. $C_{20}H_{12}$ was used at 2 μg per plate. DMSO was used at 100 μL/plate. Cultures were grown in nutrient broth for 12–14 h at 37°C on a shaking incubator. Test compounds (100 μL) were added to 500 μL of S9 mix and 100 μL of the bacterial culture. Two milliliters of top agar was added to the mixture and poured onto a plate containing minimum agar. Plates were incubated at 37°C for 48 h and his^+ revertant colonies of the *S. typhimurium* strains were manually counted. All experiments were performed in triplicate. Finally, revertant colonies were counted after 48-h incubation. Mutagenic potential of the test compound was assumed if there was a significant difference in the number of revertants of test compound with S9 mix and without S9 mix from negative control placed in *S. typhimurium* strains TA 98 and TA 100. All experiments were repeated three times, and the average values are presented.[29]

Cytotoxicity The method was based on the guidelines provided by Cayman Chemical regarding the handling of the WS-8 cell-proliferation assay kit.[30] Mouse embryonic fibroblasts (NIH 3T3), human breast cancer cells (MCF-7), human prostate cancer cells (PC-3), human colon cancer cells (HT-29), and human hepatic carcinoma cells (Hep-G2) were provided by the KCTC (Seoul, Korea) and maintained as described by Mohd-Fuat *et al.*[31] Cells were seeded in a

96-well plate at a density of 105 cells/well in 100 μL of culture medium with 100 μM of test compounds. Then, the cells were cultured in a CO_2 gas incubator at 37°C for 24–48 h. The reconstituted WST-8 mixture (10 μL) was added to each well using a repeating pipettor and gently mixed for 1 min with the help of orbital shaker until color was homogenously distributed. Cells were incubated for 2 (adherent culture) to 4 h (suspension culture) at 37°C in a CO_2 incubator. Then, the absorbance of each sample was measured using a microplate reader at a wavelength of 450 nm. Finally, the calculation of EC_{50} was carried out by applying the equation:

$$Y = \text{Bottom} + \frac{\text{TOP} - \text{Bottom}}{1 + (x/\text{EC}_{50})\,\text{Hill coefficient}}$$

where Y is the observed value, Bottom is the lowest observed value, Top is the highest observed value, and the Hill coefficient provides the largest absolute value of the slope of the curve.

Statistical Analysis All data are expressed as the means± S.D. Significant differences were conducted in pK_a and mutation examination using Student's *t*-test analysis.

RESULTS AND DISCUSSION

Kinetic Solubility The kinetic solubility of each test compound was determined using nephelometry (Table 2). Each test compound exhibited kinetic solubility, suggesting that they are soluble compounds. Diketone decursin showed the steepest kinetic solubility (173.16 μg/mL) and oxim decursin had the lowest kinetic solubility (30.98 μg/mL). Green *et al.*[17] suggested that a compound has full solubility if the kinetic solubility is >100 μg/mL), partial solubility if the kinetic solubility is 15–100 μg/mL, and poor solubility if the kinetic solubility is <15 μg/mL. In the present study, two compounds showed >100 μg/mL kinetic solubility, namely diketone decursin (173.16 μg/mL) and epoxide decursin (122.12 μg/mL, suggesting that they are highly soluble compounds. The remaining compounds exhibited kinetic solubility of 15–100 μg/mL, indicating that they are partially soluble compounds. Also, addition of diketone and epoxide as functional group to decursin proved to consists of higher kinetic solubility.

pK_a The pK_a of each test compound was determined using capillary electrophoresis and automatic titration (Table 2). The Student's *t*-test revealed significant differences in the pK_a ranges of the compounds by automatic titration from capillary electrophoresis ($p<0.05$). The pK_a determined by automatic titration showed a higher value compared with that by capillary electrophoresis for each test compound. Using both methods, decursin and decursinol angelate exhibited lowest pK_a (automatic titration: pK_a, 4.04; capillary electrophoresis: pK_a, 2.47), and rest of test compounds showed reasonable pK_a (automatic titration: pK_a, 3–4; capillary electrophoresis: pK_a, 4–6). A high degree of ionization keeps drugs out of cells and decreases systemic toxicity. A pK_a value in the range of 6–8 is advantageous for membrane penetration.[32] In the present study, all test compounds except decursin and decursinol angelate showed reasonable pK_a, suggesting that the test compounds are minimally toxic.

Log *P* The log *P* of each test compound was determined using automatic titration (Table 2). Decursinol angelate exhibited a high log *P* (4.84), and epoxide decursin had the lowest log *P* (2.09). Drugs need to be designed with the lowest log *P* to reduce toxicity and non-specific binding and to increase the ease and bioavailability of the oral formulation. Additionally, compounds are categorized by formulation through different routes of administration according to the log *P* values of the compound.[32] Thus, diketone decursin, epoxide decursin and oxim decursin could be formulated in an oral-dosage form (log *P*: 0–3). Ether decursin could be formulated for transdermal applications (log *P*: 3–4). Finally, decursin and decursinol angelate may be difficult to formulate orally (log *P*: 4–7). Thus, all test compounds except decursin and decursinol angelate exhibited a low log *P*, suggesting that they have non-specific binding, increased ease of oral formulation, and bioavailability. Also, addition of oxim, diketone, epoxide as functional group to decursin proved to be efficient for formulating an oral dosage form.

Permeability The permeability (P_{app}) value for each test compound was determined by PAMPA (Table 2). This study was conducted according to the guidelines distributed by Cyprotex discovery[13] which state that compounds with P_{app} values $<10\times10^{-6}$ cm/s are classified as poor in permeability, and those with P_{app} values $>10\times10^{-6}$ cm/s are classified as highly permeable. In the present study, no compounds exhibited P_{app} $>10\times10^{-6}$ cm/s. The highest P_{app} was obtained for Ether decursin (5.5×10^{-6} cm/s), and the lowest was exhibited by Decursinol Angelate (0.8×10^{-6} cm/s). However, the present study was conducted by applying one concentration of test compound. So, further studies are needed to measure the permeability of the test compounds exposed to Caco-2 or Madin-Darby canine kidney (MDCK) cells at different concentrations.

Acute Oral Toxicity The single-dose acute toxicity results after oral administration of 100 mg of the test compounds

Table 2. Physicochemical Characteristics of Decursin and Their Derivatives ($n=4$)

Test compounds	Kinetic solubility (μg/mL)	pK_a		log *P*	P_{app} ($\times10^{-6}$ cm/s)
		Capillary electrophoresis	Automatic titration		
Decursin	45.4±1.4	2.5±0.1	4.1±0.1**	4.1±0.8	1.7±0.1
Decursinol angelate	66.2±3.0	2.5±0.1	4.1±0.1**	4.9±0.2	0.8±0.1
Diketone decursin	173.2±6.5	4.4±0.1	5.1±0.3**	2.7±0.1	0.9±0.1
Ether decursin	53.3±4.5	3.5±0.7	4.5±0.1**	3.3±0.1	5.5±0.1
Epoxide decursin	122.1±2.2	3.6±0.1	6.7±0.1**	2.1±0.1	4.8±0.1
Oxim decursin	30.9±0.7	3.5±0.1	6.8±0.2**	2.7±0.1	1.4±0.1

Presence of significant difference in pK_a values of automatic titration from capillary electrophoresis (** $p<0.01$). P_{app}: apparent permeability.

Table 3. Single Dose Acute Toxicity after Oral administration of 100 mg Decursin and Their Derivatives in Male and Female ICR Mice for 14 d of Observation ($n=4$)

Test substance	Number of death								Weight gain
	Male				Female				
	Days								
	1	3	7	14	1	3	7	14	
Decursin	N	N	1	N	N	2	N	N	Y
Decursinol angelate	N	N	N	1	N	N	1	N	Y
Diketone decursin	N	N	N	N	N	N	1	N	Y
Ether decursin	N	N	N	1	N	N	N	N	Y
Epoxide decursin	N	1	N	1	N	1	N	1	Y
Oxim decursin	N	N	1	N	N	N	1	1	Y

N: No death.Y: Increase in weight of alive mice after 14 d of observation.

Table 4. Mutagenic Activity of Decursin and Their Derivatives in *S. typhimurium* TA 98 and TA 100 Strain in the Absence or Presence of a Metabolic-Activating Enzyme S9 ($n=3$)

S. typhimurium strains	Test compound	Number of revertants	(Mean±S.D.)	NC	PC
TA 98	Decursin	Without S9 mix	20±2	15±2	280±18***
		With S9 mix	25±5	27±8	483±21
	Decursinol angleate	Without S9 mix	18±6	14±1	280±17***
		With S9 mix	25±1	26±8	483±21
	Diketone decursin	Without S9 mix	35±5	38±2	261±19***
		With S9 mix	30±3	36±6	461±22
	Ether decursin	Without S9 mix	25±5	28±2	243±15**
		With S9 mix	33±11	34±11	342±28
	Epoxide decursin	Without S9 mix	502±33**	22±1**	220±18**
		With S9 mix	91±2**	38±5	330±14
	Oxim decursin	Without S9 mix	115±5**	23±1**	224±18**
		With S9 mix	68±10*	37±5	333±14
TA100	Decursin	Without S9 mix	128±5	139±2	540±47*
		With S9 mix	142±22	143±21	849±134
	Decursinol angleate	Without S9 mix	139±1	139±2	540±47*
		With S9 mix	144±8	143±21	849±134
	Diketone decursin	Without S9 mix	138±13	151±12	691±41**
		With S9 mix	137±11	147±6	1019±79
	Ether decursin	Without S9 mix	156±8	165±10*	708±9*
		With S9 mix	171±4	188±10	992±149
	Epoxide decursin	Without S9 mix	1433±97**	156±4*	608±10***
		With S9 mix	240±24*	172±7	851±25
	Oxim decursin	Without S9 mix	288±11**	156±4*	608±10***
		With S9 mix	405±10**	172±7	851±25

All test compounds were 5000 μg/plate. NC: Negative control. PC: Positive control. NC: DMSO (100 μL/plate). PC: $C_{13}H_8NO_2$ for TA 98 without S9 mix (1 μg/plate), NaN_3 for TA 100 without S9 mix (1 μg/plate), $C_{20}H_{12}$ for TA 98 and TA 100 with S9 mix (2 μg/plate). Presence of significant difference in number of revertants with S9 mix and without S9 mix from NC (*$p<0.05$, **$p<0.01$). Presence of significant difference in number of revertants in each chemicals without S9 mix from with S9 mix (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

to ICR mice are shown in Table 3. A single dose was applied following the guidelines provided by Pucaj *et al.*[26] The results revealed weight gain in both the male and female ICR mice after 14 d of observation. The highest number of male ICR mice deaths was observed among those receiving epoxide decursin (days 3 and 14: 1 death/d). The highest number of deaths among female ICR mice were observed for decursin, epoxide decursin, and oxim decursin (2 deaths each). Overall, epoxide decursin was the most toxic (four deaths total), whereas diketone decursin and ether decursin exhibited less toxic effects (1 death total). Addition of epoxide as functional group to decursin proved to consists of oral toxic effect. However, further studies are needed to measure the toxic effects at different doses, routes of administration, animals, and lab parameters.

Mutation The mutagenic activity of the test compounds in *S. typhimurium* TA 98 and TA 100 are shown in Table 4. A large number of TA98 and TA 100 revertants was observed for epoxide decursin without the S9 mix (502, 1433). Epoxide decursin showed the highest number of revertants with S9 mix in TA 98 (91), whereas oxim decursin exhibited the highest number of revertants in TA 100 with the S9 mix (405). A *t*-test comparing the compounds with and without the S9 mix from negative control revealed no significant difference in the

Table 5. Cytotoxic Effect of 100 μM Decursin and Their Derivatives in Mouse and Human Cells after 4 h (n=3)

Test substances	EC_{50} (μM)				
	NIH 3T3	MCF-7	PC-3	HT-29	Hep-G2
Decursin	>100	>100	>100	>100	>100
Decursinol angelate	>100	>100	>100	>100	>100
Diketone decursin	>100	>100	>100	>100	>100
Ether decursin	57.9±2.0	>100	>100	36.1±1.0	4.9±2.0
Epoxide decursin	6.42±3.0	>100	>100	>100	>100
Oxim decursin	>100	>100	>100	>100	>100

NIH 3T3: mouse embryonic fibroblast. MCF-7: human breast cancer cell. PC-3: human prostate cancer cell. HT-29: human colon cancer cell. Hep-G2: human hepatic carcinoma cell.

number of revertants, except for epoxide decursin and oxim decursin; thus, epoxide and oxim decursin compounds have only mutagenic activity. So, addition of epoxide and oxim functional group to decursin suggests of consisting mutagenic effects. Previous studies suggest that there are different ways of detoxification pathway for epoxide.[33,34] However, no studies have been conducted regarding detoxification of epoxide and oxim decursin. Further investigation should be conducted regarding detoxification pathway for epoxide and oxim decursin, different cells, and concentrations of the test compounds.

Cytotoxicity The EC_{50} cytotoxic effects of the test compounds in mouse and human cells after 4 h of exposure are shown in Table 5. All test compounds exhibited an EC_{50} >100 μM except ether and epoxide decursin. Ether decursin had an EC_{50} of 57.9 μM in mouse embryonic fibroblast (NIH 3T3) cells, 36.1 μM in human colon cancer cells (HT-29), and 4.92 μM in human hepatic carcinoma cells (Hep-G2). Additionally, epoxide decursin exhibited an EC_{50} of 6.42 μM in mouse embryonic fibroblast (NIH 3T3) cells, suggesting a toxic effect from these compounds. Thus, except for ether and epoxide decursin, all test compounds could provide a safe therapeutic effect. Also, addition of ether and epoxide as functional group to decursin suggests of consisting cytotoxic effects. However, the present study was conducted by applying one concentration of test compound. So, further studies are needed to measure the cytotoxicity of the test compounds at different doses.

CONCLUSION

We successfully conducted a physicochemical and toxicity study of decursin, decursinol, and their derivatives. Decursin and decursinol angelate may be difficult to formulate into an oral dosage form. Ether decursin was toxic and could only be administered transdermally. Additionally, epoxide and oxim decursin were also toxic. However, diketone decursin exhibited high solubility, a reasonable pK_a range, and less toxicity and could be administered orally. There are no previous studies on the pharmacokinetic effects of diketone decursin. Thus, diketone decursin is potentially valuable compound that should be investigated.

Acknowledgements This research was supported by Samnam Pharmaceuticals and the Korea Research Institute of Chemical and Technology.

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